Plant Breeding **126**, 240—243 (2007)
doi:10.1111/j.1439-0523.2007.01345.x

Rapid phenotypic assessment of bird cherry-oat aphid resistance in winter wheat

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With 2 tables

Received September 26, 2005/Accepted October 20, 2006
Communicated by F. Ordon

Abstract

Rhopalosiphum padi L. causes significant damage to winter wheat (*Triticum aestivum* L.), even without obvious aboveground symptoms of injury. Our objectives were to develop a juvenile-plant bioassay for bird cherry-oat aphid (BCOA) resistance that allows rapid phenotypic differentiation. Central features of the bioassay include root and shoot weight measurements of 3-week-old seedlings produced in seed germination pouches, a 14-day aphid exposure period, and a non-infested control treatment to establish a baseline for expected biomass per genotype. Cultivars used in bioassay development were 'Illinois Rustproof' and 'Skala', which showed smaller BCOA-induced reductions in biomass than the more susceptible genotypes, 'Patrick' and 'Scout 66'. Mean reductions in root biomass were 48% for 'Patrick' and 'Scout 66', compared with 29% for 'Illinois Rustproof' and 'Skala'. This rapid and repeatable bioassay is extendable to large wheat collections and inbred line populations.

Key words: *Triticum aestivum* — *Rhopalosiphum padi* — resistance — germplasm

The bird cherry-oat aphid (BCOA) reduces root and shoot growth in wheat (Riedell et al. 2003) and causes significant loss of grain yield (Pike and Schaffner 1985, McPherson et al. 1986, Riedell et al. 1999) without obvious aboveground visual symptoms of injury. The BCOA is often the dominant cereal aphid in winter wheat during the fall and may persist until the soil surface freezes (Kieckhefer and Gustin 1967). Direct injury from BCOA feeding is attributable to depletion of phloem nutrients or to toxin injection (Hsu 1963). Bird cherry-oat feeding, which may occur throughout plant development from seedling to tillering stages, also may affect winter wheat cold hardiness by depleting fructans in crown tissue (Wellso et al. 1985). Indirect injury can be even more devastating if BCOA acts as a vector for Barley yellow dwarf virus (BYDV) (Riedell et al. 1999). Detrimental effects associated with BCOA exposure may be exacerbated when winter wheat is planted early as a dual-purpose crop (Royer et al. 2005).

Mullins (1993) found that using imidacloprid, which exhibits both systemic and contact insecticidal functions, could be used in BCOA management strategies. Others have found antibiosis and tolerance to BCOA in some wheat accessions (Kazemi and Emden 1992, Papp and Mesterhazy 1993, 1996, Lamb and MacKay 1995). Given that genetic variation for BCOA resistance exists within common wheat, a protocol should be developed to allow discrimination amongst experimental lines typically evaluated in a breeding programme. Critical to this protocol is the capability to observe chronic effects of BCOA feeding on root and shoot growth, in contrast to bioassays for other aphids with acute effects and qualitative segregation of genotypic effects (Starks and Burton 1977).

Baker et al. (1997) suggested that transparent seedling growth pouches could be used to observe shoot and root growth differences between BCOA-infested and non-infested treatments. Our objective was to optimize their procedure to develop a rapid and quantitative juvenile-plant bioassay for BCOA resistance.

Materials and Methods

Plant materials: Three experiments were conducted in controlled-environment chambers (Conviron Environments Limited, Winnipeg, MB) using two pairs of genotypes of wheat, *Triticum aestivum* L. with putative differences in BCOA resistance: 'Illinois Rustproof' (resistant) vs. 'Patrick' (susceptible), and 'Skala' (resistant) vs. 'Scout 66' (susceptible). These two pairs were observed to have divergent reactions to BCOA feeding in previous studies.

Seedling growth conditions: Some procedural components were common to all experiments. Seeds were germinated and seedlings were grown in seed germination pouches, 16.4-cm wide × 17.7-cm tall (Mega International, Minneapolis, MN). Five uniform size kernels were placed with the crease down in each pouch and covered with sterilized sand. Two holes, 7 mm in diameter, were punched in the bottom of each pouch to allow pouch hydration. Ten pouches were placed in a rack that was immersed in a 5.4-l container containing 2 l tap water and 0.4 ml azoxystrobin (fungicide), plus 0.2 ml imidacloprid if the designated rack of pouches was assigned a non-infested treatment (control). The water level was allowed to rise approximately 4 cm above the bottom of the pouches. Preliminary tests showed that treatment with imidacloprid did not affect seedling growth (2003, data not shown). To minimize any potential border effects, each set of 10 pouches was surrounded on each end with a border pouch treated similarly but not used in subsequent measurements. The containers were placed in growth chambers (interior area of 1.4 m, 185 PPFD) at 21°C.

Experiment description: Aphid colonies were clonally derived from a single source and maintained on 'Jagger' wheat. They were confirmed by ELISA to be non-viruliferous for BYDV. Based on work by Baker et al. (1997), 1 week after initiation of germination, infested 'Jagger' leaves containing 40–60 aphids per leaf were placed over the pouches for a target infestation level of 10–15 aphids per seedling. Aphids migrated from the dying Jagger leaf onto the test plants and began feeding and reproducing. Aphid numbers quickly increased to very high levels per seedling, resulting in high selective pressure necessary to efficiently differentiate resistant from

susceptible seedling reactions. The addition of 0.2 ml imidacloprid to the control treatment prevented migration of aphids onto non-infested plants. Root and shoot dry weight (48 h at 65°C) were determined after removing all aphids. Response variables for all experiments were root and shoot dry weight of viable plants, reported as weight per plant.

For Experiment 1, aphid infestation periods of 10, 12, 14, 16 and 18 days were evaluated for cultivars 'Illinois Rustproof' and 'Patrick' at 21°C. A corresponding non-infested treatment was applied for intervals equivalent to the infestation period. Each cultivar was assigned to separate pouches, arranged as five pairs per rack. Multiple containers were used to accommodate the treatment × infestation period combinations. The experiment was repeated over time to provide two replications. The mean biomass of three to five plants per pouch and five pouches for each treatment period per replicate constituted the experimental unit in the statistical analysis.

For Experiment 2, 'Illinois Rustproof' and 'Patrick' were re-evaluated using the optimized bioassay to further evaluate their response to a 14-day BCOA infestation period. Each cultivar was represented by five pouches (10 pouches per container) in one of two containers receiving either the infested treatment or the non-infested treatment. The experiment was repeated to provide two replicates, with an experimental unit defined as above for one treatment period. Experiment 3 was conducted similar to Experiment 2 but with additional replication, and cultivars 'Skala' and 'Scout 66' were substituted for 'Illinois Rustproof' and 'Patrick'. One trial consisted of two containers (10 pouches per container with five pouches per genotype) for each treatment. Four consecutive trials were conducted over time. Mean biomass of three to five plants per pouch and five pouches per replicate comprised an experimental unit in the statistical analysis.

Statistical analysis: For Experiments 1 and 2, genotype means were reported as least-square means generated by the MIXED procedure of SAS (SAS Institute 2001) applied to each treatment separately, with replicates considered a random factor. Type 3 tests of fixed effects provided *F*-values for determining significance of days (duration of infested or non-infested treatment), genotype ('Illinois Rustproof' vs. 'Patrick'), and their interaction, where appropriate. For Experiment 3, an analysis of variance was conducted across trials, with trials and replicates within trials considered random, to test for significance of trial × treatment and trial × genotype × treatment interactions. Each trial was then analyzed separately to generate least-square estimates of genotype × treatment means. Pairwise contrasts of 'Skala' vs. 'Scout 66' within treatments were made using a *t*-test with the Tukey–Kramer adjustment.

Results

In Experiment 1, we examined five infestation periods spanning 10–18 days to determine if the two reference genotypes, 'Illinois Rustproof' and 'Patrick', could be differentiated on the basis of root or shoot biomass with shorter or longer aphid exposure than our original period of 14 days. In the infested treatment alone, 'Illinois Rustproof' and 'Patrick' responded similarly across infestation periods, as evidenced by a non-significant genotype effect and genotype × infestation period interaction (Table 1). Only in the non-infested treatment were differences detected at $P = 0.09$ (root biomass) or at $P = 0.01$ (shoot biomass). One distinctive feature in the root biomass data was a tendency for 'Illinois Rustproof' to maintain greater relative biomass after 12–16 days infestation as a proportion of its biomass accumulated after the same number of days in the non-infested treatment.

'Illinois Rustproof' and 'Patrick' were re-evaluated in Experiment 2 with greater subsampling per genotype in each replication than in Experiment 1. These genotypes again responded similarly in the infested treatment following a 14-day exposure (data not shown), but relative to their biomass in the non-infested treatment, 'Patrick' suffered a greater reduction in root biomass (48%) than 'Illinois Rustproof' (27%). Their differential responses were validated by a significant genotype × treatment interaction ($P = 0.01$). A similar pattern was observed for shoot biomass, in which the respective reductions equalled 31% and 17%, but the genotype × treatment interaction was not significant ($P = 0.34$).

The same bioassay was applied to a different pair of genotypes, 'Skala' and 'Scout 66', again chosen on the basis of visual differences in root elongation following BCOA exposure in germination pouches. Four sequential trials were conducted in Experiment 3 to examine consistency of response amongst assays. Absolute differences between genotypes went undetected for root and shoot biomass in the infested treatment (Table 2), as with 'Patrick' and 'Illinois Rustproof'. Relative to biomass produced in the non-infested treatment, however, 'Scout 66' suffered a greater reduction in root (47%) and shoot biomass (37%, both averaged across four trials) than did the more resistant genotype, 'Skala' (30% reduction for root biomass and 23% for shoot biomass). Differential responses of 'Scout 66' and 'Skala' to aphid damage were validated by significant genotype × treatment interactions detected in all,

Table 1: Seedling root and shoot dry weight (mg per plant) for two wheat genotypes exposed to bird cherry-oat aphid for five infestation periods

	Roots						Shoots					
	Infested			Non-infested			Infested			Non-infested		
Period[1] (days)	Illinois Rustproof	Patrick	$\bar{x}$	Illinois Rustproof	Patrick	$\bar{x}$	Illinois Rustproof	Patrick	$\bar{x}$	Illinois Rustproof	Patrick	$\bar{x}$
10	8.0	7.9	8.0	12.3	6.3	9.3	27.0	26.3	26.6	39.3	38.8	39.0
12	6.5	5.9	6.2	13.5	13.8	13.6	28.4	25.0	26.7	51.5	37.9	44.7
14	6.4	8.8	7.6	9.4	14.5	12.0	29.4	30.4	29.9	44.0	44.4	44.2
16	9.7	3.3	6.5	20.0	16.3	18.2	32.7	25.0	33.8	63.4	52.3	57.8
18	7.8	5.0	6.4	20.8	13.7	17.2	28.8	23.8	26.3	60.0	51.7	55.8
Mean	7.7	6.2	6.9	15.2	12.9	14.1	29.3	28.5	27.7	51.6	45.0	48.3
LSD (0.05)												
Period		4.7			4.4			7.1			6.6	
Genotype		3.0			2.8			4.5			4.2	
Genotype × period		6.7			6.2			10.0			9.3	

[1]Duration of aphid exposure in the infested treatment, or equivalent number of days biomass accumulation occurred in the non-infested treatment.

Table 2: Seedling root and shoot dry weight (mg per plant) of 'Skala' and 'Scout 66' in the presence or absence of bird cherry-oat aphid (14-day exposure) using the established protocol in four tandem trials

		Experiment 1		Experiment 2		Experiment 3		Experiment 4	
Treatment	Genotype	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
Infested	Skala	12.9	27.8	11.7	22.5	10.9	21.4	11.1	20.4
	Scout 66	11.4	28.5	9.0	25.2	9.3	24.4	10.5	23.9
	t-test, adj. P-value[1]	0.54	0.99	0.10	1.00	0.11	0.93	0.94	0.40
	t-value, absolute	1.35	0.35	2.45	0.00	1.56	0.56	0.55	1.60
Non-infested	Skala	16.3	32.5	16.4	31.8	16.2	26.5	18.0	29.2
	Scout 66	17.8	43.1	17.7	38.6	21.1	38.9	19.9	40.2
	t-test, adj. P-value[1]	0.50	< 0.001	0.56	0.01	< 0.001	< 0.001	0.25	< 0.001
	t-value, absolute	1.38	8.75	1.32	3.87	4.78	7.06	1.93	7.18
Genotype × treatment (P-value)		0.06	< 0.01	0.01	0.02	< 0.01	< 0.01	0.10	< 0.01

[1]Based on Tukey–Kramer adjustment.

but two of the eight *F*-tests for shoot and root biomass amongst the four trials. The lack of trial × treatment ($P = 0.16$, roots; $P = 0.35$, shoots) and trial × genotype × treatment interactions ($P = 0.23$, roots; $P = 0.31$ shoots) indicates conclusions drawn from this series of experiments should be repeatable.

Discussion

A bioassay that establishes a time–course relationship with duration of infestation might provide a more complete assessment of resistance to feeding, but such an assay would not be practical for screening large germplasm collections or breeding populations. Our earlier attempts to establish this assay indicated visual differences amongst random bread wheat accessions could be detected in root elongation after about 2 weeks infestation (Baker et al. 1997). This bioasssay may be used to detect genotypic differences effectively and rapidly in resistance to BCOA feeding (approximately a 2-week period), but detection hinges upon a non-infested treatment to establish a baseline for expected biomass produced by a given genotype.

We have since extended the bioassay to several groups of breeding lines from the U.S. Great Plains, with a typical trial containing 30–50 genotypes and each genotype represented by a single pouch and two containers (replicates) in both aphid-infested and non-infested treatments. Genetic variation for biomass was detected in the absence of aphid feeding, which again confounded differences in actual biomass in the infested treatment alone. Simple correlations between treatments were generally not significant or moderately low. Hence, greater biomass in the non-infested treatment did not necessarily produce greater biomass in the infested treatment, as may be expected. Information gained from both treatments should be considered simultaneously, such as a ratio of infested-to-control biomass, to more accurately assess BCOA resistance. Although aphid feeding is restricted entirely to leaf tissue, the severity of BCOA injury is not tissue-dependent. Depending on the group of materials evaluated, simple correlation coefficients for the biomass ratio for shoots vs. roots varied from 0.15 ($P > 0.05$) to 0.80 ($P < 0.01$). When the objective is to identify the highest level of resistance, we recommend evaluation of BCOA resistance based on damage assessment of both shoot and root growth.

In summary, this protocol provides a relatively rapid (3 weeks), repeatable bioassay for BCOA resistance in wheat that has utility in breeding and germplasm evaluation programmes. Essential to effective discrimination amongst genotypes is a non-infested control treatment for baseline estimates of differential growth rate and biomass accumulation. Given the resources available in this study relative to growth chamber space, number of seedling pouches, and container size, as many as 200 non-replicated genotypes could be managed by one person, including four replicates of three reference genotypes as a source of experimental error for statistical tests if required. A more rapid assessment might be achieved by visual classification of root and shoot biomass relative to a control treatment, if the primary objective is only to identify genotypes either highly susceptible or highly resistant to BCOA feeding.

Acknowledgements

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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